Fine Structural Studies of Leukocytes from Patients and Heterozygotes with the Chediak-Higashi Syndrome

By Steven D. Douglas, Ralph S. Blume and Sheldon M. Wolff

The Chediak-Higashi Syndrome (CHS) is characterized by large abnormal cytoplasmic organelles in cells including leukocytes which contain lysosome-like structures. This rare disease is inherited as an autosomal recessive disorder, and the clinical features include partial oculocutaneous albinism, frequent and severe pyogenic infections, and the development of a lymphoma-like (accelerated) phase, with pancytopenia, hepatosplenomegaly, lymphadenopathy, and widespread mononuclear cell infiltrates. Affected patients usually die in early childhood with pyogenic infections, or less commonly, hemorrhage.

Based on morphologic and histochemical studies of peripheral blood leukocytes, some investigators have considered the large abnormal granules or "inclusions" to be giant lysosomes. Such morphologic abnormalities are also present in cells from the Aleutian mink (CHS mink), a strain of partial albino Hereford cattle (CHS cattle), and a strain of Beige mice (CHS mice). Similar abnormal organelles have been observed in the skin, ocular pigment epithelium, hair, peripheral nerve, scent glands, adrenal, pituitary and the organs of the GI tract of affected animals or children.

Fine structural studies of the Chediak-Higashi syndrome in man have been limited. A systematic fine structural study of peripheral blood leukocytes from four patients with the Chediak-Higashi syndrome, their parents (heterozygotes), and siblings was undertaken. Particular emphasis has been placed on the pleomorphic substructure of the abnormal lysosome-like organelles, their frequency and distribution, and their presence in the heterozygous state.

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In all illustrations, cell pellets were fixed in 1.5 per cent glutaraldehyde followed by post fixation in osmium tetroxide, en bloc, stained with 1 per cent uranyl acetate and embedded in Araldite, Sections were stained with lead citrate.

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MATERIALS AND METHODS

Clinical Material

Four patients with the Chediak-Higashi syndrome hospitalized at the National Institutes of Health were available for study: three males: LeR, age 17; LaR, age 16 (sibling of LeR); SP, age 6, and one female: TH, age 6. Certain of the clinical features of the three males have been published previously by others.10,11 Two patients (SP and TH) were in drug induced remissions of the accelerated phase. The six parents of the patients were also studied, as were three apparently normal siblings of patient SP.

Tissue Culture

A long-term suspension culture of lymphoid cells derived from peripheral leukocytes of patient SP was examined. The details of the development and characterization of this established cell line have been reported elsewhere.12

Electron Microscopy

Fifteen ml. of peripheral blood was drawn into disposable plastic syringes containing 5 units heparin per milliliter. Buffy coats were obtained by sedimentation and centrifuged at 500 rpm. for 30 minutes at 20 C. The cell pellets were fixed with 1.5 per cent glutaraldehyde in 0.1 M phosphate buffer13 for 2–4 hours. The cells were post-fixed in phosphate buffered osmium tetroxide, dehydrated and embedded in Araldite. Some specimens were stained en bloc with 1 per cent aqueous uranyl acetate for two hours at 20 C. following post-fixation in osmium tetroxide. Ultramicrotomy was performed on an LKB Ultrotome using glass or diamond knives. Thin sections were stained with uranyl acetate and lead citrate14 and examined in an RCA-EM-3G electron microscope.

RESULTS

Patients

General cytologic features. Electron microscopy of the peripheral blood of the four patients reveals the presence of large granules (0.5–2.5 μ in diameter) in the lymphocytes, monocytes, and cells of the granulocytic series. The percentage of cells in which these abnormal organelles were observed varies as a function of cell type. However, the frequency, distribution and characteristics of these granules in any one cell type were similar in all four patients.

Lymphocytes. The peripheral blood lymphocytes of patients with the Chediak-Higashi syndrome show many of the cytoarchitectural features of normal cells.15 Although the normal lymphocyte has little cytoplasm and few cytoplasmic organelles, the paucity of these organelles is even more striking in lymphocytes from these patients (Figs. 1 and 3). However, some cells have bizarre nuclear lobulation (Fig. 5) and/or an alteration of chromatin to a predominantly euchromatic pattern with prominent nucleoli (Figs. 7 and 10). Frequently, the cytoplasmic contents are limited to a few single ribosomes, a few mitochondria, and a single giant lysosome-like organelle (Figs. 1–4,8). The mitochondria of many cells which contained giant lysosome-like organelles often had a swollen appearance (Figs. 5–7), however, this may be due to fixation.

The giant lysosome-like organelles, present in 40–60 per cent of lymphocyte sections, are usually elliptical in shape and about 1.0–2.0 μ in long diameter. They often contain electron dense, 0.1–0.3 μ in diameter spherical structures which are not membrane bound (Figs. 2 and 4), and in addition, large fene-
Fig. 1.—Peripheral blood lymphocyte from Patient SP. The cell contains a single giant lysosome-like organelle. Note the paucity of other cytoplasmic organelles. \( \times 22,000 \).

Fig. 2.—High power micrograph of the giant organelle in Figure 1. The structure is single membrane bound and contains several electron dense ovoid structures (0.1-0.3 \( \mu \text{m} \) in diameter) as well as a large fenestrated lipid body. \( \times 90,000 \).
Fig. 3.—Lymphocyte from Patient SP showing giant lysosome-like organelle. × 22,000.

Fig. 4.—Higher power micrograph of organelle shown in Figure 3. Note the electron dense ovoid structures and fenestrated lipid bodies within the organelle. Crystalloid material with a spacing of 150 Å is also seen in the matrix of the organelle. × 73,000.
Stratified structures with varying degrees of osmiophilia are present (Figs. 2 and 4). Some of the lysosome-like organelles contain a matrix of crystalloid material with a periodicity of 150 Å (Fig. 4), while others contain small vesicular structures (0.1–0.3 µm in diameter) (Fig. 6). Small 0.2–0.4 µm diameter typical myelin-like figures are observed infrequently (Fig. 9). No distinct relationship between these abnormal organelles and other cytoplasmic components is apparent. Virus-like particles were not observed in the nuclei or cytoplasm of these cells.

Granulocytes. Polymorphonuclear neutrophils from patients with the Che- diak-Higashi syndrome contain both the specific and azurophil granules characteristic of this cell type (Figs. 11–14). A characteristic small Golgi apparatus is seen; nuclear morphology is normal, and nuclear pores are frequently observed. Many of the cell sections (approximately 85 per cent) contain one or more giant lysosome-like organelles. In contrast to the structures found in lymphocytes, these organelles usually contain amorphous material varying in electron density (Fig. 14) as well as sheets of membrane-like structures and whorls of membranous material (Fig. 11) differing from the typical myelin figures which are occasionally present. Giant membrane-bound areas of cytoplasm 1.0–2.5 µm in diameter, which contain finely granular osmiophilic material, are present (Fig. 12) as has been previously described. No virus-like particles are seen.

Parents and siblings. A small number of lymphocytes, about 1–5 per cent of cells examined, from the peripheral blood of each of the six parents (heterozygotes) contained large (0.6–1.2 µm in diameter) organelles similar to those observed in the patients' lymphoid cells (Figs. 15–18). Structures of this size were not observed in sections of normal peripheral blood lymphocytes which usually contain 2–6 heterogeneous, electron-dense (0.3–0.6 µm diameter) single membrane bound bodies. No abnormal organelles were observed in the granulocytes or monocytes from the parents. Peripheral blood leukocytes from three healthy siblings of one patient (SP) showed no detectable fine structural abnormalities.

Established Cell Line

Electron microscopic study of a suspension culture lymphoid cell line established from the peripheral blood of patient SP demonstrates that the predominant cell type is a large lymphoblast-like cell with a euchromatic nucleus and a prominent nucleolus. The cytoplasm contains numerous ribosomes, strands of rough surfaced endoplasmic reticulum, frequent glycogen particles, and mitochondria often swollen with loss of cristae (Figs. 19–22). Reticular arrays of particles are observed and will be reported in detail elsewhere. About 10 per cent of the cells contain large single membrane bound lysosome-like organelles (0.7–2.0 µm in diameter) (Figs. 21 and 22) which usually contain fibrillar material and intensely electron dense atypical myelin figures. Structures of this size are observed in other lymphoid cell cultures only very infrequently.

Discussion

Previous reports have emphasized the presence of giant single membrane
Fig. 5.—Peripheral blood lymphocytoid cell from Patient TH. Nuclear lobulation is shown. Giant lysosome-like structure at upper right. X 10,500.

Fig. 6.—Lymphocytoid cell from TH showing single large organelle at upper left. This structure contains many small vesicles. X 12,000.

Fig. 7.—Lymphocytoid cell from peripheral blood of TH. The nucleolus is promi-
bound organelles in cells of patients with the Chediak-Higashi syndrome and the possible lysosomal nature of these granules. However, little attention has been paid to the pleomorphism of substructures of these organelles, the possible existence of similar structures in heterozygotes, and to the cytoarchitectural characteristics of the cells in which they appear.

The present studies demonstrate a diverse spectrum of giant single membrane bound lysosome-like organelles in the leukocytes of affected patients. In lymphocytes, the contents of these cytoplasmic organelles include: fenestrated structures, crystalloid material with a period of 150 A, vesicular structures varying in osmiophilia, small electron dense spherules (0.1-0.3 μ in diameter), lamellar stacks and myelin-like figures. In granulocytes, they contain: amorphous material, lamellated sheets, whorls of membranous structures, typical myelin figures, and in addition, the previously demonstrated giant membrane bound areas of cytoplasm with finely granular osmiophilic material. The lysosome-like organelles in circulating lymphocytes are distinct from those in granulocytes or in cultured lymphoid cells. In contrast to the lipoidoses in which the abnormal lysosomal contents represent an accumulation of a specific metabolic product, the internal substructure of the giant CHS granule is primarily a function of cell type.

The lysosome-like architectural alterations observed in Chediak-Higashi lymphoid cells in suspension culture (atypical myelin figures) are not qualitatively unique. Although usually smaller than those present in the Chediak-Higashi lymphoid cells in culture, myelin-like figures occur in lymphoid cell cultures of man or animals without this genetic defect and can also be induced by chloroquine or toxic environmental conditions. The difference observed between CHS lymphoid cells in vivo and in vitro would support the concept that the formation of these abnormal organelles is in part determined by the external as well as the internal cellular environment. The fine structural changes seen in CHS are similar to the cytolysosomes, residual bodies, and lipofuscin granules which appear in cells during the aging process and in damaged cells in vitro.

A small percentage of lymphoid cells with abnormal lysosome-like organelles are present in the peripheral blood of all CHS heterozygotes. These exhibit the substructure of the abnormal organelles of lymphoid cells observed in CHS homozygotes. The identification of these abnormal lysosome-like organelles in all heterozygotes may permit morphologic detection of the heterozygous state and demonstrates that the defect is expressed in the heterozygous state. The large single membrane bound organelle contains dense osmiophilic material and vesicular structures. × 11,000.

Fig. 8.—Peripheral blood lymphocyte from Patient SP. A giant lysosome-like organelle is present at upper right. × 16,000.

Fig. 9.—Lysosome-like structure from lymphocyte of LeR. Within this structure are bodies of varying osmiophilia. A myelin-like whorl is present at the right. × 35,000.

Fig. 10.—Portion of a lymphocytoid cell from LeR with a giant organelle near its center. Note the difference in heterochromatin distribution between this cell and the cell in Figure 8. × 16,000.
Fig. 11.—Peripheral blood polymorphonuclear neutrophil from TH. Azurophil and specific granules are present. In addition, note several large membrane bound bodies containing whorls of membranous material. × 14,000.

Fig. 12.—Polymorphonuclear neutrophil from LaR. The cytoplasm contains large membrane bound organelles which contain finely granular osmiophilic material. × 9,000.
Fig. 13.—Polymorphonuclear neutrophil from LeR. Note typical azurophil and specific granules. A large membrane bound body is present at the upper right. × 9,000.

Fig. 14.—Membrane bound body seen in Figure 13 at higher magnification. Note whorls of membranous lamellae and heterogeneity of contents. × 40,000.
Fig. 15.—Peripheral blood lymphocyte from RR, mother of LaR and LeR. A single large membrane bound organelle is present surrounded by four smaller lysosome-like structures. × 12,000.

Fig. 16.—Higher power micrograph of organelle shown in Figure 15. × 44,000.

Fig. 17.—Small blood lymphocyte from HR, father of LaR and LeR. Two large membrane bound organelles are present. × 16,000.

Fig. 18.—Another large membrane bound organelle present in lymphocyte from HR. Note heterogeneity of lipid-like contents. × 35,000.
Fig. 19.—Lymphoblastoid cell from established cell line derived from peripheral blood of Patient SP. The nucleolus is prominent and chromatin distribution predominantly euchromatic. A single large organelle is present in the Golgi region at upper center. $\times 9,000$.

Fig. 20.—Portion of another lymphoblastoid cell from the cell line established from SP. Note giant organelle and surrounding smaller membrane bound organelles. $\times 14,000$.

Fig. 21.—Higher power micrograph of organelle from cell in SP culture. Note electron dense myelin-like structures within this organelle. $\times 26,000$.

Fig. 22.—Higher power micrograph of organelle present in the cell shown in Figure 20. Myelin-like structures and heterogeneity of contents are evident. $\times 35,000$. 

gotes in vivo. The low frequency of the lymphoid cells containing abnormal granules would certainly explain the variable results of previously reported attempts to demonstrate them in heterozygotes.\textsuperscript{22} The paucity of heterozygous cells in which abnormal granules are present and the small number of these organelles per cell differs from the situation in fibroblasts cultured in vitro from CHS heterozygotes.\textsuperscript{23}

The present study of peripheral blood from four patients, two with documented mononuclear cell tissue infiltrates of the accelerated phase of the Chediak-Higashi syndrome, does not demonstrate widespread autophagy or cytoplasmic sequestration in circulating leukocytes in contrast to a previous report.\textsuperscript{1} No evidence of giant organelles inducing destruction of the surrounding cytoplasm was seen. Consequently, the present data does not support the contention that the membranes of these giant granules are “leaky” or abnormally fragile.\textsuperscript{4} Moreover, the giant granules in neutrophils of both CHS mink and humans have been shown to be more stable to the stimulus of phagocytosis than normal neutrophil granules.\textsuperscript{24,25} An explanation for these differences is not readily apparent. In addition, no evidence was found to support the association of virus-like particles with this syndrome in general or the accelerated phase in particular as was previously reported.\textsuperscript{26} Herpes-like particles have been found in leukocytes of normal healthy subjects and in a variety of other illnesses,\textsuperscript{27} but were not seen in any of our patients including the two with the accelerated phase.

Whether or not the morphologic defect in CHS reflects a predisposition to early cell death remains to be established. The relationship between the genetic defect, morphologic findings and predisposition to pyogenic infections is unknown, as is the mechanism of giant granule formation. The present study demonstrates the presence of several types of large abnormal granules in peripheral blood leukocytes from patients with CHS and moreover, that their substructure differs in the various cell types. These findings suggest that the fundamental defect in CHS may well not involve either an abnormality of membrane content or of lysosome formation, but rather, an as yet undefined biochemical defect. Further investigation of the biochemical properties of these organelles in each cell type is essential in order to fully assess the fundamental functional derangements in this syndrome.

**Summary**

Fine structural studies of leukocytes from four patients with the Chediak-Higashi syndrome, including two in the accelerated phase, demonstrate a pleomorphism of substructure of the giant lysosome-like organelles pathognomonic of this syndrome. These organelles differ in composition in relationship to the cell type in which they occur. Many circulating lymphoid cells have altered nuclear chromatin and a diminution in cytoplasmic constituents. Virus-like particles are not seen and there is no evidence of abnormal cytoplasmic sequestration. Large abnormal lysosome-like organelles are present in a small percentage of lymphoid cells from the six parents of these patients, demonstrating that heterozygotes exhibit a cellular abnormality in vivo.
FINE STRUCTURAL STUDIES

SUMMARIO IN INTERLINGUA

Studios microstructural de leucocytos ab quatro patientes con le syndrome Chediak-Higashi—incluse duo in le phase accelerate—ha demonstrate un pleomorphismo del sub-structura in le gigante organellas lysosomoide que es pathognomonic de iste syndrome. Iste organellas differe in lor composition in relation al typo cellular in le qual illos occurre. Multe circulante celularis lymphoide ha alterate chromatina nucleari e un diminution in le constituentes cytoplasmatic. Nulle particulas simile a virus es vidite e nulle evidentia es trovate de anormal sequestration cytoplasmatic. Large anormal organellas lysosomoide es presente in un basse procentage de cellulas lymphoide ab le sex parentes studiate de iste patientes, lo que demonstra que heterozygotes exhibe in vivo un anormalitate cellular.

REFERENCES

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